Steroidal Sapogenins. X.[1] Qualitative Color Test for Pseudosapogenins

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The key step in transformation of steroidal sapogenins to pregnane derivatives is conversion to the so-called "pseudosapogenin" acetates,[2] *i.e.*, 20(22)-furostenol acetates. Because of the difficulty of crystallization of the products and the lack of significant detail in their infrared spectra, the extent of completion of the pseudomerization reaction may be followed, in a general way, by disappearance of the characteristic spiroketal absorption bands of the starting material,[3] rather than by the appearance of any properties characteristic of the product.

We have discovered that when the Tortelli–Jaffé color reaction[4,5] is applied to pseudosapogenins, a blue color is formed differing from the typical green color produced by ditertiary bridgehead ethylenic bonds or by olefins isomerizable to this type. The development of the blue color is apparently specific for the pseudosapogenin structure and does not occur with unsaturated steroids having double bonds at C_5–C_6, C_9–C_{11}, C_3–C_4, C_{16}–C_{17}, with dihydropseudosapogenins or with dihydrosapogenins. We have not tested pseudosapogenins with ditertiary, bridgehead ethylenic bonds.

The ultraviolet spectrum of the blue-colored material produced in the test shows maxima at 307 and 607 mμ (Beckman spectrophotometer). Bromine is transparent at 607 mμ at the concentrations used. Attempts to adapt the reaction for quantitative measurements were unsuccessful because the intensity of color developed reaches a maximum with about 1 mg. of steroid (in 10 ml. total volume) and is lower with quantities below and above this concentration. Color development does not follow the Beer–Lambert law. We have found the test to be particularly useful as an indication of completeness of reaction in pseudosapogenin transformations, *e.g.*, hydrogenation, oxidation, and the like.

Experimental

The color test was carried out in several ways. In the most sensitive method, the sample, 1 mg. of pseudosapogenin in 1 ml. of commercial C.P. chloroform, was diluted with 5 ml. of glacial acetic acid and mixed with 1 ml. of 0.1% bromine in chloroform. The mixture was underlayered with 0.1 ml. of 1% bromine in chloroform and after 30 minutes was diluted to 10 ml. with acetic acid and mixed.

For a rapid but less sensitive procedure, the Tortelli–Jaffé test as modified by Heilbron and Spring[5] gave a blue zone at a position intermediate between the liquid–liquid interface and the surface. The color developed within 5 minutes using amounts of pseudosapogenins over 0.3 mg. in the volume recommended. The compounds tested are listed below.

Positive color reaction:

- 20(22)-Furosten-26-ol
  (3-desoxy-pseudosarsasapogenin)
  (3-desoxy-pseudosmilagenin)
- 5α–20(22)-Furostene-3β,26-diol-12-one
  (pseudohecogenin)
- 5,20(22)-Furostadiene-3β, 26-diol
  (pseudodiosgenin)
- 20(22)-Furostene-2,3β,26-triol
  (pseudomarkogenin)
  (pseudosamogenin)
- 5α–20(22)-furostene-3β, 26-diol
  (pseudotigogenin)

Negative color reaction:

- 16,22-Epoxy-20ξ-cholestane-3β,26-diol
  (dihydropseudotigogenin)
- 16,22-Epoxy-20ξ, 22ξ-coprostane-3β,26-diol
  (dihydropseudosarsasapogenin)
- 16,22-Epoxy-22b-coprostan-26-ol
  (dihydro-3-desoxysarsasapogenin)
- 3β,16-Dihydroxy-allopregnan-20-one 16-(5-acetoxy-4-methyl valerate) (tigone)
- 22a-Spirosta-3,5-diene
- 5-Spirostenes and acetates
- 5α,22a-Spirost-9-(11)-en-3β-ol
  (9-dehydrohecogenin)
- Saturated sapogenins
- Saturated 3-desoxysapogenins
- 3β,26-Dihydroxy-cholest-5-ene-16,22-dione
  (kryptogenin)
- 3β,26-Dihydroxy-furost-16(23)-en-21-one
  (fesogenin)
- 16-Pregnene-3,20-dione
- 16-Allopregnene-2α,3β-diol-20-one diacetate
- 16-Allopregnen-3β-ol-20-one acetate
- Cholestan-3β-ol
- Cholesterol
- Stigmasterol
- Progesterone

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